SEQUENCE LISTING

<110> Theide, Mark
Flake, Alan

<120> In Utero Transplantation of Human Mesenchymal Stem Cells

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<170> PatentIn 3.2

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PCR Analysis: To screen the ovine tissues for the *presence* of human cells, total cellular DNA was subjected to PCR analysis for human specific ß-2 microglobulin using a modification of previously described methods (Gilliland, et al., Proc. Nat. Acad. Sci., Vol. 87, pgs. 2725-2729 (1990)). In brief, 1ug of total cellular DNA isolated from the above-mentioned tissues was added to individual 0.65mL microcentrifuge tubes and placed on ice. A master mix was prepared and added on ice such that the final concentration of reagents for each sample was 2.5U Amplitaq Gold DNA polymerase (Perkin Elmer, Norwalk, CT), 200uM deoxytriphosphates (dNTP's, Pharmacia, Piscataway, NJ), 50mM KCl, 10mM Tris-C1 (pH 8.3 at 22°C), 1.5mM $MgCl_2$, 0.01% gelatin, and 1uM upstream and downstream primers. Specific primers for human ß-2 microglobulin were selected based on the published human sequence (D), (upstream primer 5′-GTGTCTGGGTTTCATCAATC (SEQ ID NO: 1), downstream primer 5′-GGCAGGCATACTCATCTTTT (SEQ ID NO: 2), and shown to amplify specifically human, not ovine, DNA. The samples were kept on ice until the thermocycler block reached 95°C, when the samples were placed immediately into the block for 9 minutes. Samples were amplified for 50 cycles of 30 seconds at 94°C followed by 30 seconds of primer annealing at 55°C followed by 1 minute of extension at 72°C. Upon completing the final cycle, samples were incubated for 5 minutes at 72°C. PCR products were subjected to electrophoresis through a 2.5% NuSieve/1% Seakern agarose gel containing 0.5ug ethidium bromide/mL in 1X Tris acetate running buffer. The gels were illuminated with UV 280-nm light and photographed with type 55 positive/negative Polaroid film.